(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 438 474 B1

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
15.05.1996 Bulletin 1996/20

(21) Application number: **89911584.4**

(22) Date of filing: **12.10.1989**

(51) Int Cl.[6]: **C12P 21/08**, C12N 15/85, C12N 5/10, C12N 15/13

(86) International application number:
PCT/GB89/01207

(87) International publication number:
WO 90/04036 (19.04.1990 Gazette 1990/09)

(54) PRODUCTION OF ANTIBODIES FROM TRANSGENIC ANIMALS

ERZEUGUNG VON ANTIKÖRPERN AUS TRANSGENEN TIEREN

PRODUCTION D'ANTICORPS A PARTIR D'ANIMAUX TRANSGENIQUES

(84) Designated Contracting States:
AT BE CH DE FR GB IT LI LU NL SE

(30) Priority: **12.10.1988 GB 8823869**

(43) Date of publication of application:
31.07.1991 Bulletin 1991/31

(73) Proprietors:
- **MEDICAL RESEARCH COUNCIL**
  London W1N 4AL (GB)
- **THE BABRAHAM INSTITUTE**
  Babraham, Cambridge CB2 4AT (GB)
- **BRUGGEMANN, Marianne**
  Cambridge CB2 6SA (GB)

(72) Inventors:
- **BRUGGEMANN, Marianne**
  Cambridge CB2 1NA (GB)
- **SURANI, Azim, M.**
  Cambridge CB4 3AH (GB)
- **NEUBERGER, Michael, Samuel**
  Cambridge CB5 8TE (GB)

(74) Representative: **Matthews, Heather Clare et al**
Keith W Nash & Co
Pearl Assurance House
90-92 Regent Street
Cambridge CB2 1DP (GB)

(56) References cited:
EP-A- 0 264 166
WO-A-88/00239
WO-A-88/01648
WO-A-88/05077
WO-A-88/10118

- **TIBTECH, vol. 5, 1987; A.J. CLARK et al., pp. 20-24/**
- **TIBTECH, vol. 5, 1987; R.B. CHURCH, pp. 13-19/**
- **SCIENCE, vol. 240, 1988; R. JAENISCH, pp. 1468-1474/**
- **TRENDS IN GENETICS, vol. 1, 1985; F.W. ALT et al., pp. 231-236/**
- **NATURE, vol. 323, 1986; E. ROBERTSON et al., pp. 445-448/**
- **ADVANCES IN GENETICS, vol. 24, 1987; G. SCANGOS et al., pp. 285-316/**

EP 0 438 474 B1

Printed by Jouve, 75001 PARIS (FR)

Description

Field of Invention

This invention concerns the production of antibodies (or immunoglobulins).

Background to the Invention

It is known to produce transgenic animals capable of producing foreign proteins, including immunoglobulins, in body fluids such as milk (see Clark et al., Tibtech (1987) 5, 20-24 and Church et al., Tibtech (1987) 5, 13-19). Scangos et al., Advances in Genetics (1987) 24, 285-316 and Alt et al., Trends in Genetics (1985) 1, 231-236 are both review articles which include references to work in which functionally rearranged immunoglobulin genes are introduced into mice. Jaenisch, Science (1988) 240, 1468-1474 is a review of transgenic animals and their uses. The section on page 1471 under the heading "Immune System" refers, inter alia, to papers of Goodhardt et al. (Proc. Natl. Acad. Sci, USA (1987) 84, 4229-4233) and Bucchini et al (Nature (1987) 326, 409-411) concerning rearrangement of rabbit or chicken immunoglobulin light chain genes introduced to mice in germline configuration.

Summary of the Invention

According to one aspect of the present invention there is provided a method of producing an immunoglobulin, comprising inserting into the germline of a non-human animal DNA that includes a nucleic acid segment coding for at least part of an immunoglobulin of human origin which includes a gene segment coding for a human immunoglobulin heavy chain region wherein said gene segment is not in fully rearranged form, such that the DNA is rearranged in the animal to encode a repertoire of immunoglobulins with part or parts derived from the inserted DNA and expressed in cells or body fluid of the animal; and obtaining immunoglobulin from cells or suitable body fluid of the animal.

In a further aspect the invention provides a method of producing an immunoglobulin to a particular antigen, comprising producing a transgenic animal by inserting into the germline of the animal DNA of foreign origin coding for at least a part of an immunoglobulin of human origin which includes a gene segment coding for a human immunoglobulin heavy chain region, such that said DNA of foreign origin undergoes rearrangement or mutation in the lymphoid tissue of the transgenic animal to produce a variety of rearranged genes that encode immunoglobulins, immunoglobulin fragments or immunoglobulin chimeric molecules, such that, following challenge of the transgenic animal with a particular antigen, the immunoglobulin to the antigen that is encoded by the rearranged or mutated DNA is expressed in cells or body fluid of the animal; and obtaining immunoglobulin from cells or suitable body fluid of the animal.

DNA of foreign origin means DNA derived from a different animal source. For example where the transgenic animal is a mouse, the inserted DNA is of non-mouse, e.g. human, origin.

The inserted DNA may be produced from an animal source, or may be produced synthetically. The DNA may code for at least part of a known immunoglobulin or may be modified to code for at least part of an altered immunoglobulin. Suitable techniques for these processes are well known.

The inserted DNA may be expressed in the transgenic animal, resulting in production of an immunoglobulin derived at least in part from the inserted material. It is found the inserted DNA is rearranged in the transgenic animal, so that a repertoire of immunoglobulins with part or parts derived from inserted genetic material may be produced even if the inserted material is incorporated in the germline in the wrong position or with the wrong geometry. Depending on the nature of the inserted material, the animal may produce a chimaeric immunoglobulin, e.g. of mixed mouse/human origin, where the material of foreign origin encodes only part of the immunoglobulin, or the animal may produce an entirely foreign immunoglobulin, e.g. of wholly human origin, where the DNA of foreign origin encodes an entire immunoglobulin. Potentially therapeutically useful immunoglobulins suitable for use with humans thus may be produced by use of the invention.

Polyclonal antisera may be produced from the transgenic animal following immunisation. Alternatively, monoclonal antibodies may be produced from the transgenic animal, eg by fusing spleen cells from the animal with myeloma cells and screening the resulting hybridomas to select those producing the desired antibody. Suitable techniques for such processes are well known to those skilled in the art.

In an alternative approach, the DNA may be incorporated in the animal in such a way that the desired antibody is produced in body fluids such as serum or external secretions of the animal, such as milk, colostrum or saliva. For example, by inserting in vitro DNA encoding for at least part of an immunoglobulin of foreign origin into a gene of a mammal coding for a milk protein and then introducing the gene to a fertilised egg of the mammal, eg by injection, the egg may develop into an adult female mammal producing milk containing immunoglobulin derived at least in part from the inserted DNA. The desired antibody may then be harvested from the milk. Suitable techniques for carrying out such processes are known to those skilled in the art.

Another possibility involves removal from the animal of immunoglobulin-producing cells generated by the animal after insertion of genetic material, followed by in vitro selection of cells producing an immunoglobulin of interest. The immunoglobulin can then be produced in vitro from the selected cells in known manner.

It has been found that a transgenic animal can produce chimaeric or foreign immunoglobulin (derived from inserted DNA) in response to an immunogen subsequently introduced to the animal. Accordingly, by introducing foreign, eg human, DNA encoding for substantially the entire species specific regions of an immunoglobulin it may be possible to stimulate the animal to produce foreign immunoglobulin to any antigen introduced to the animal. The transgenic animal could thus provide a highly useful, convenient and valuable source of human immunoglobulins to a large range of antigens.

It is thought that it may be important for the inserted DNA to be integrated in proximity on the genome for successful rearrangement. The inserted DNA may thus be in the form of DNA cloned into prokaryotic vectors such as plasmids and cosmids. Multiple plasmids or cosmids may also be used, but it is probably necessary for these to integrate in proximity on the genome. It may also prove possible to insert larger DNA fragments by using yeast artifical chromosome vectors (see Burke, D T, Carle, G F and Olson, M V (1987) "Cloning of large segments of exogenous DNA into yeast by means of artifical chromosome vectors" Science, 236, 806-812), or by introduction of chromosome fragments (see Richer, J and Lo, C W (1989) "Introduction of human DNA into mouse eggs by injection of dissected human chromosome fragments" Science 245, 175-177). Vertebrate chromosome or DNA fragments may also be used as the source of the inserted DNA.

The inserted DNA may be introduced to the host in conventional manner, for example by injection or other procedures into fertilised eggs or embryonic stem cells.

It may be convenient to use a host animal that initially does not carry genetic material encoding immunoglobulin constant regions so that the resulting transgenic animal will use only the inserted foreign genetic material when producing immunoglobulins. This can be achieved either by using a naturally occuring mutant host lacking the relevant genetic material, or by artificially making mutants eg in cell lines ultimately to create a host from which the relevant genetic material has been removed.

Where the host animal carries genetic material encoding immunoglobulin constant regions, the transgenic animal will carry the naturally occuring genetic material and the inserted genetic material and will produce immunoglobulins derived from the naturally occuring genetic material, the inserted genetic material and mixtures of both types of genetic material. In this case the desired immunoglobulin can be obtained by screening hybridomas derived from the transgenic animal, eg by exploiting the phenomenon of allelic exclusion of antibody gene expression or differential chromosome loss.

In a further aspect the present invention produces a transgenic animal, particularly a non-human mammal, which has had inserted into its germline DNA that includes a nucleic acid segment coding for at least part of an immunoglobulin of human origin which includes a gene segment coding for a human immunoglobulin heavy chain region wherein said gene segment is not in fully rearranged form, such that the DNA is rearranged in the animal to encode a repertoire of immunoglobulins with part or parts derived from the inserted DNA and expressed in cells or suitable body fluid of the animal.

The invention also includes within its scope an immunoglobulin obtainable from a transgenic animal in accordance with the invention or produced by the method of the invention.

In one example, lines of transgenic mice were established, carrying a DNA segment introduced into their germline that contains germline-configuration immunoglobulin VH genes, some of the D segments, and all the JH and Cmu gene segments. One of the VH genes, all the JH segments and the exons encoding the secreted heavy-chain constant region of IgM antibody were of human origin, with the remaining material being of mouse origin. The gene segments undergo productive VH-D-JH joining in the lymphoid tissue of the transgenic mice, with resultant synthesis of human/mouse chimaeric IgM antibody in serum.

Following immunisation, hybridomas have been established by fusion between spleen cells from the transgenic mice with the NSO myeloma. Many of the hybrids secerete human chimaeric IgM monoclonal antibodies. These lines of transgenic mice can therefore be used for the production of chimaeric human antisera or monoclonal antibodies.

Further, the mice make a response following immunisation with human antigens, producing chimeric antibodies to introduced antigen, and this approach should therefore also prove useful for the production of a repertoire of conventional human or chimaeric human antibodies directed against human as well as heterologous antigens, as the transgenic mice will not be tolerant to human antigenic determinants.

In another example, transgenic mice carrying exclusively human VH, D, JH and C mu sequences were produced by injecting into fertilised mouse eggs cosmids containing human VH genes, D segments, J segments and the C mu constant region. Resulting mice produced between 10 and 100 ug/ml antibody containing human mu chains in their serum, with mouse IgM being present at about 200 ug/ml.

The invention will be further described, by way of illustration, in the following Examples which refer to the accompanying drawings, in which:

Figure 1 illustrates the structure of plasmid DNA;

Figure 2 is a Southern blot analysis of DNA from tissues and from hybridomas derived from transgenic mice with the DNA of Figure 1 incorporated into the germline;

Figure 3 is a Northern blot analysis of cytoplasmic RNA from hybridomas from the transgenic mice;

Figure 4 is an analysis of immunoglobulin secreted by two of the hybridomas established from the transgenic mice;

Figure 5 illustrates the structure of IgA lambda plasmid DNA;

Figure 6 is a series of FACS histograms showing fluorescence intensity of a fixed number of cells plotted against cell number;

Figure 7 is a series of FACS profiles showing fluorescence intensity of a fixed number of cells plotted against scatter, with each dot representing a cell; and

Figure 8 is a further series of FACS histograms similar to Figure 6.

EXAMPLE 1

The Transgene

Plasmid DNA with the structure shown in Figure 1 was injected into mouse eggs. In this Figure human sequences are represented by filled bars; mouse sequences by unfilled bars; vector by cross-hatched bars, and D elements by reverse hatched bars. Restriction endonuclease cleavage sites are abbreviated as follows: Bg, BglII; P, PvuI; R, EcoRI; K, KpnI; B, BamHI; X, XbaI; H, HindIII.

The provenance of the constituent DNA segments is as follows:

The Vector:

The entire "mini-IgH locus" was cloned between the EcoRI and BglII site of a pUCI2 derivative that contains BglII linkers cloned into a filled-in NarI site.

The VH genes:

There are two VH genes in the mini-locus. The VH26 is of human origin and was obtained as a BglII-EcoRI 0.85 Kb fragment from phage lambda VH26 [Matthyssens & Rabbitts (1980) PNAS 77, 6561-6565]. The VH-186.2 is a germline VH gene from C57BL/6 mice and was obtained as 2.5 Kb SacI-KpnI fragment [Bothwell, Paskind, Reth, Imanishi-Kari, Rajewsky & Baltimore (1981) Cell 24, 625-637].

The D segments: the BALB/c mouse D-052 element was

obtained as a 221 nt XhoI-SacI fragment. Following cloning between the SacI and SalI sites of MI3tgl31, site directed mutagenesis with either oligonucleotide

5'-GCGTCACCGTGGTAGCTGCTACCGTAGTAATAAACACTGTGGTCC or

5'-GCGTCACCGTGGTCGTAACCATAGTAGACACTGTGGTGC

was used to create MI3tgl31 clones carrying D elements related to those of the mouse SP2 and FL16 families, respectively. These D families are found in both mouse and human. Another D element - the human D-052 - was included within the human JH cluster (see below).

The JH cluster:

A 3.5 Kb BglII fragment from human DNA was used that includes the six functional human JH segments, three pseudo JHs as well as the human D-Q52 element [Ravetch et al. (1981) Cell 27, 583-591].

The IgH Enhancers:

Part of the human IgH enhancer is included within the BglII fragment containing the JH cluster. A full copy of the mouse enhancer is included within the 1 Kb XbaI fragment.

The Switch region and Cmu region:

The 7.5 Kb XbaI fragment of human DNA includes the mu switch region and exons 1 to 4 of the mu heavy-chain constant region. The mu membrane exons and the bulk of the intron between the Cmu4 exon and the CmuMI membrane exon are provided by a 2.5 Kb HindIII-SphI fragment of the mouse mu CH gene in which the SphI site was converted to a BglII site by use of linkers.

The Transgenic mice

Plasmid DNA was linearised with BglII, purified after electrophoresis in an agarose gel and injected into the male pronucleus of fertilised eggs of C57BL/6J x CBA/Ca mice as previously described [Reik et al. (1987) Eur. J. Immunol. 17, 465-469]. Southern blot analysis of tail DNA revealed that 12 of the 32 mice born carried the mini-locus. Most subsequent work was performed on offspring of three founder mice - Hlg 17, 19 and 29 all of which carry a low number (2-5) of copies of the mini-locus.

Serum Assays

Serum of the founder mice was tested by ELISA for the presence of antibody containing antigenic determinants characteristic of human IgM. The uniminunised transgenic mice proved to contain tween 10 and 100 ug/ml of chimaeric human IgM in their serum. Immunofluorescence analysis of lymphocytes in peripheral blood also revealed the presence of cells staining with biotinylated species-specific anti-human Ig M antibody and fluorescein-conjugated streptavidin.

Hybridomas from transgenic mice

Transgenic mice were immunised intraperitoneally with either human red blood cells or sheep red blood cells. Spleens were removed at various times after immunisation, fused with the NSO myeloma and hybrids selected in HAT medium. Many of these hybrids made chimaeric human IgM as revealed by ELISA assay.

DNA Rearrangement of the mini-locus

Southern blot analysis of DNA from tissues from the transgenic mice as well as from the hybridomas revealed that there is a high frequency of DNA rearrangements within the mini-Ig locus in the lymphoid tissue of the transgenic mice.

DNA from tissues or hybridomas established from the transgenic mice was digested with EcoRI and hybridized with a human IgH enhancer probe (BalI-BglII fragment) that hybridizes to the region between the human J6 element and the mouse IgH enhancer in the mini-locus. The results of Southern blot analysis of the DNA are shown in Figure 2. The sizes in Kb of marker fragments are given in the Figure.

Transcription of the mini-locus

Cytoplasmic RNA (5 ug) from the NSO fusion partner or from hybridomas from the transgenic mice was probed with human Cmu, human VH26 or mouse VH186 probes The results of Northern blot analysis of the cytoplasmic RNA are shown in Figure 3, which reveals that the hybridomas contained mRNA that hybridized with probes for human mu as well as for either or both of the VH26 or VH186 V genes. Thus both the human VH26 and mouse VH186 are able to rearrange and thus create a cell-line that secretes a chimaeric human IgM antibody.

Antibody secretion by hybridomas from the transgenic mice

Protein production by cloned hybridoma cell-lines was analysed by use of biosynthetic labelling with L-[^{35}S] methionine and subsequent purification with anti-human mu antiserum. In particular, cells were incubated overnight in medium containing L-[^{35}S] methionine and IgM antibody purified from the culture supernatant by immunoprecipitation and an anti-human mu antiserum. The purification from the supernatants of the transgenic hybridomas 35.5 and 24a was performed in the presence of a large excess (50ug) of non-radioactive, purified mouse monoclonal IgM antibody (B1-8) as indicated by "+" in Figure 4. As seen using the mouse IgM secreting cell-line, the anti-human mu antiserum

cross-reacts with mouse mu but this cross-reaction can be competed by nonradioactive mouse BI-8 IgM antibody - see the four lanes on the left of Figure 4.

This illustrates that it is possible to establish hybridomas from these transgenic mice that secrete antibodies with different human mu chains.

EXAMPLE 2

Transgenic animals can be created that produce specific antibodies in both their body fluids and external secretions. By way of illustration, this example concerns transgenic mice that carry integrated into their germline the genes encoding the heavy and light chains of an antigen-specific chimaeric human IgA2 antibody.

Nine transgenic mouse lines were established that carried germline integrations of the DNA illustrated in Figure 5. In Figure 5, the thick filled lines depict mouse Ig DNA, the hatched lines human DNA, open boxes the mouse IgH and SV 40 enhancers, and thin filled lines the pSV2gpt vector. Restriction site abreviations are as in Figure 1. The plasmid is a derivative of pSV-VNPH alpha 2 described previously [Bruggemann et al, J. Exp. Med (1987) 166, 1351-1361] and contains a 7.4 kb EcoRI fragment including the rearranged lambda 1 gene of the mouse HOPC2020 plasmacytoma [Bernard et al (1978), Cell 15, 1133-1140]. Plasmid DNA was linearised at the PvuI site in the vector and transgenic mice derived as previously described [Reik et al, Eur. F. Immunol (1987) 17, 465-469].

The transgenic IgA 2 lambda antibody has specificity for the hapten 4-hydroxy-3-nitrophenacetyl (NP). Expression of the transgenic antibody was measured by ELISA assay using NP-bovine serum albumin coupled to the plastic plate and developing with a biotinylated anti-human alpha antiserum. Total Ig was measured using anti-mouse Ig coated plastic and developing with biotinylated anti-mouse kappa antiserum. The concentration of chimaeric anti-NP IgA2 was determined in the colostrum, serum and milk from seven transgenic mice and one control mouse, and the resuts are given in Table 1. Colostrum was taken from the mother following hormonal injection within 24 hours of giving birth and milk was taken from the mother when the litter was 13-15 days old; serum was obtained at the same time as milk.

From these data it is clear that transgenic animals can be used for specific antibody production thus allowing large scale production from milk, colostrum, sera, saliva etc as well as allowing the breeding of animals that yield a milk that is dosed with specific beneficial antibodies. It is also clear that the concentration of transgenic antibody is higher in colostrum than in milk. Moreover, the presence of the transgene does not affect the ability of the animal to make a large amount of endogenous antibody. The animals show no signs of being significantly immunodeficient or unhealthy.

Example 3

In this example, antigen-specific hybridomas were produced using human mu chains from transgenic mice.

To show that the transgenic mice described in Example 1 can be used to produce antigen-specific antibodies in which the heavy-chain contribution to the antigen-combining site is provided by the transgenic human heavy-chain minilocus, the transgenic mice were immunised with 10^7 sheep red cells (SRC). Spleen cells were fused 6 days later with the NSO plasmacytoma (Köhler and Milstein, Nature, 256, 495-497, 1975). Cells were plated out in 96-well costar plates such that the expected seeding frequency was 1 hybridoma per well. Human mu positive hybrids were detected in an ELISA using biotinylated anti-human IgM. Antigen-specific hybrids have been identified in haemagglutination using sheep red cells. Wells were chosen that contained antibodies specific for sheep red cells and that contained human mu but neither mouse mu nor mouse gamma heavy chains as determined by ELISA.

To demonstrate that the antibody secreted by the selected hybridomas was indeed a human mu/mouse light chain anti-SRC immunoglobulin, analysis in a fluorescence-activated cell sorter (FACS) was used. For FACS analysis, 10^7 sheep red cells were incubated with 20 ul culture supernatant for 30 min, washed with phosphate-buffered saline once and further incubated with either biotinylated anti-human mu heavy chain antiserum, biotinylated anti-mouse mu antiserum or biotinylated anti-mouse kappa light chain anti-serum. After 30 min cells were washed as before and incubated with FITC coupled streptavidin. Cells were washed after 30 min and after gentle disruption were ready for the anaylsis.

Results for 3 different hybrids producing IgM directed against the sheep red cell antigen are shown in the FACS histograms of Figures 6, 7 and 8, respectively.

The letters A, B, C and D in these Figures denote different staining. Figures 6A, 7A, 8A are results for negative controls using sheep red cells alone, sheep red cells incubated with antibody or sheep red cells incubated with fluorescinated (FITC) second antibody (either anti-human mu anti-mouse mu, anti-mouse kappa). Figures 6B, 7B, 8B are results using sheep red cells incubated with hybrid culture supernatant and fluoresceinated (FITC) anti-human mu. Figures 6C, 7C, 8C are results using sheep red cells incubated with culture supernatant from the transgenic hybrids and fluoresceinated (FITC) anti-mouse mu. Figure 6D is results using sheep red cells incubated with hybrid culture supernatant and fluoresceinated (FITC) anti-mouse kappa.

In Figures 6 and 8 fluorescence intensity of a fixed number of cells is plotted against cell number. In Figure 7 fluorescence intensity of a fixed number of cells is plotted against scatter, with each dot representing a cell.

A shift of the profiles to the right (increased fluorescence) denotes a positive stain that can only been seen for antibodies containing human mu heavy chains and mouse kappa light chains but not for antibodies containing mouse mu heavy chains or mouse gamma heavy chains (not shown).

Table 1

Antibody in the body fluids of IgA2, lambda 1-mice					
	IgA2 anti NP Ab			Total kappa bearing Ab	
Mouse	Serum	Milk	Colostrum	Milk	Colostrum
TG1	10	0.6	2.1	960	600
TG2	6.3	0.56	1.4	1000	420
TG3	11.3	1.3	ND	735	ND
TG4	7.3	0.8	1.4	780	660
TG5	30	7.6	10.0	1250	ND
TG6	34.6	5.0	10.0	500	600
TG7	6.3	0.64	0.93	780	600
Control	0	0	0	1136	660
ND, not determined					

All concentration in ug/ml

Claims

1. A method of producing an immunoglobulin, comprising inserting into the germline of a non-human animal DNA that includes a nucleic acid segment coding for at least part of an immunoglobulin of human origin which includes a gene segment coding for a human immunoglobulin heavy chain region wherein said gene segment is not in fully rearranged form, such that the DNA is rearranged in the animal to encode a repertoire of immunoglobulins with part or parts derived from the inserted DNA and expressed in cells or body fluid of the animal; and obtaining immunoglobulin from cells or suitable body fluid of the animal.

2. A method of producing an immunoglobulin to a particular antigen, comprising producing a transgenic non-human animal by inserting into the germline of the animal DNA of foreign origin coding for at least part of an immunoglobulin of human origin which includes a gene segment coding for a human immunoglobulin heavy chain region, such that said DNA of foreign origin undergoes rearrangement or mutation in the lymphoid tissue of the transgenic animal to produce a variety of rearranged genes that encode immunoglobulins, immunglobulin fragments or immunoglobulin chimeric molecules such that, following challenge of the transgenic animal with a particular antigen, the immunoglobulin to the antigen that is encoded by the rearranged or mutated DNA is expressed in cells or body fluid of the animal; and obtaining immunoglobulin from cells or suitable body fluid of the animal.

3. A method according to claim 1 or 2, wherein polyclonal antiserum comprising the immunoglobulin is obtained from the animal.

4. A method according to claim 1 or 2, wherein monoclonal antibody comprising the immunoglobulin is produced using cells obtained from the animal.

5. A method according to claim 1 or 2, wherein the immunoglobulin is produced in a body fluid or secretion of the animal.

6. A method according to claim 1 or 2, wherein the immunoglobulin is produced in vitro from cells obtained from the animal.

7. A method according to any one of the preceding claims, wherein the inserted DNA encodes substantially the entire species specific regions of an immunoglobulin.

8. A method according to any one of the preceding claims, wherein the transgenic animal is a mouse.

9. A method according to any one of the preceding claims wherein the inserted DNA comprises a plasmid or cosmid; multiple plasmids or cosmids; a yeast artificial chromosome; or vertebrate chromosome or DNA fragments.

10. A method according to any one of the preceding claims, wherein the DNA is inserted by injection or other procedures into fertilised eggs or embryonic stem cells.

11. A method according to any one of the preceding claims, wherein the animal initially does not carry genetic material encoding immunoglobulin constant regions.

12. An immunoglobulin of foreign origin obtainable by the method of any one of the preceding claims.

13. A transgenic non-human animal which has had inserted in its germline DNA that includes a nucleic acid segment coding for at least part of an immunoglobulin of human origin which includes a gene segment coding for a human immunoglobulin heavy chain region wherein said gene segment is not in fully rearranged form, such that the DNA is rearranged in the animal to encode a repertoire of immunoglobulins with part or parts derived from the inserted DNA and expressed in cells or suitable body fluid of the animal.

Patentansprüche

1. Verfahren zur Herstellung eines Immunglobulins, umfassend die Insertion von DNA in die Keimlinie eines nicht-menschlichen Tieres, die ein mindestens einen Teil eines Immunglobulins menschlichen Ursprungs codierendes Nucleinsäuresegment umfaßt, das ein Gensegment einschließt, das einen Bereich der schweren Kette eines menschlichen Immunglobulins codiert, wobei das Gensegment nicht in vollständig rearrangierter Form vorliegt, wobei die DNA in dem Tier rearrangiert wird, so daß sie eine Anzahl von Immunglobulinen mit einem Teil oder Teilen, die von der inserierten DNA stammen, codiert, und in den Zellen oder der Körperflüssigkeit des Tieres exprimiert wird; und die Gewinnung von Immunglobulin aus Zellen oder geeigneter Körperflüssigkeit des Tieres.

2. Verfahren zur Herstellung eines Immunglobulins gegen ein bestimmtes Antigen, umfassend die Produktion eines transgenen nicht-menschlichen Tieres durch Insertion von DNA fremden Ursprungs in die Keimlinie des Tieres, die mindestens einen Teil eines Immunglobulins menschlichen Ursprungs codiert, das ein einen Bereich der schweren Kette eines menschlichen Immunglobulins codierendes Gensegment einschließt, wobei die DNA fremden Ursprungs einem Rearrangement oder einer Mutation im Lymphoidgewebe des transgenen Tieres unterliegt, so daß eine Vielzahl von rearrangierten Genen, die Immunglobuline, Immunglobulinfragmente oder chimere Immunglobuline codieren, produziert werden, so daß, nach Exposition des transgenen Tieres mit einem bestimmten Antigen, das Immunglobulin gegen das Antigen, das durch die rearrangierte oder mutierte DNA codiert wird, in Zellen oder Körperflüssigkeit des Tieres exprimiert wird; und Gewinnung von Immunglobulin aus Zellen oder geeigneter Körperflüssigkeit des Tieres.

3. Verfahren nach Anspruch 1 oder 2, wobei das Immunglobulin umfassendes polyclonales Antiserum von dem Tier erhalten wird.

4. Verfahren nach Anspruch 1 oder 2, wobei das Immunglobulin umfassende monoclonale Antikörper unter Verwendung von von dem Tier erhaltenen Zellen produziert werden.

5. Verfahren nach Anspruch 1 oder 2, wobei das Immunglobulin in einer Körperflüssigkeit oder einem Sekret des Tieres produziert wird.

6. Verfahren nach Anspruch 1 oder 2, wobei das Immunglobulin in vitro aus vom Tier erhaltenen Zellen produziert wird.

7. Verfahren nach einem der vorangehenden Ansprüche, wobei die inserierte DNA im wesentlichen die gesamten Speziesspezifischen Bereiche eines Immunglobulins codiert.

8. Verfahren nach einem der vorangehenden Ansprüche, wobei das transgene Tier eine Maus ist.

9. Verfahren nach einem der vorangehenden Ansprüche, wobei die inserierte DNA ein Plasmid oder Cosmid, multiple Plasmide oder Cosmide, ein künstliches Hefechromosom oder Vertebratenchromosom oder DNA-Fragmente umfaßt.

10. Verfahren nach einem der vorangehenden Ansprüche, wobei die DNA durch Injektion oder andere Verfahren in befruchtete Eier oder embryonale Stammzellen inseriert wird.

11. Verfahren nach einem der vorangehenden Ansprüche, wobei das Tier zunächst kein genetisches Material trägt, das konstante Bereiche von Immunglobulin codiert.

12. Immunglobulin fremden Ursprungs, erhältlich durch das Verfahren nach einem der vorangehenden Ansprüche.

13. Transgenes nicht-menschliches Tier, in dessen Keimlinie DNA inseriert ist, die ein mindestens einen Teil eines Immunglobulin menschlichen Ursprungs codierendes Nucleinsäuresegment umfaßt, das ein Gensegment einschließt, welches einen Bereich der schweren Kette eines menschlichen Immunglobulins codiert, wobei das Gensegment nicht in vollständig rearrangierter Form vorliegt, wobei die DNA in dem Tier rearrangiert wird, so daß sie eine Anzahl von Immunglobulinen mit einem Teil oder Teilen, die von der inserierten DNA stammen, codiert, und in Zellen oder geeigneter Körperflüssigkeit des Tieres exprimiert wird.

Revendications

1. Procédé de production d'une immunoglobuline, comprenant l'insertion dans la lignée germinale d'un animal non-humain d'un fragment d'ADN qui contient un segment d'acide nucléique codant pour au moins une partie d'une immunoglobuline d'origine humaine, incluant un segment de gène codant pour une région de chaînes lourdes d'immunoglobuline humaine, dans lequel ledit segment de gène n'est pas sous une forme entièrement réarrangée, tel que l'ADN soit réarrangé dans l'animal pour coder un répertoire d'immunoglobulines avec une ou des parties dérivée(s) de l'ADN inséré et exprimée(s) dans les cellules ou un fluide corporel de l'animal ; et l'obtention de l'immunoglobuline à partir des cellules ou du fluide corporel approprié de l'animal.

2. Procédé de production d'une immunoglobuline dirigée contre un antigène particulier, consistant à produire un animal transgénique non-humain en insérant dans la lignée germinale de l'animal un fragment d'ADN d'origine étrangère codant pour au moins une partie d'une immunoglobuline d'origine humaine incluant un segment de gène codant pour une région de chaîne lourdes d'une immunoglobuline humaine, tel que ledit ADN d'origine étrangère subisse un réarrangement ou une mutation dans le tissu lymphoide de l'animal transgénique pour produire une variété de gènes réarrangés qui codent des immunoglobulines, des fragments d'immunoglobulines ou des molécules chimériques d'immunoglobulines tel que, après provocation de l'animal transgénique avec un antigène particulier, l'immunoglobuline dirigée contre l'antigène qui est codée par l'ADN réarrangé ou ayant subi une mutation s'exprime dans les cellules ou un fluide corporel approprié de l'animal.

3. Procédé selon la revendication 1 ou 2, dans lequel l'antisérum polyclonal comprenant l'immunoglobuline est obtenu de l'animal.

4. Procédé selon la revendication 1 ou 2, dans lequel l'anticorps monoclonal comprenant l'immunoglobuline est produit en utilisant les cellules obtenues de l'animal.

5. Procédé selon la revendication 1 ou 2, dans lequel l'immunoglobuline est produite dans un fluide corporel ou une sécrétion de l'animal.

6. Procédé selon la revendication 1 ou 2, dans lequel l'immunoglobuline est produite in vitro à partir de cellules obtenues de l'animal.

7. Procédé selon l'une quelconque des revendications qui précèdent, dans lequel l'ADN inséré code essentiellement pour les régions spécifiques de la totalité des classes d'une immunoglobuline.

8. Procédé selon l'une quelconque des revendications qui précèdent, dans lequel l'animal transgénique est une souris.

9. Procédé selon l'une quelconque des revendications qui précèdent, dans lequel l'ADN inséré comprend un plasmide ou un cosmide ; des plasmides ou des cosmides multiples ; un chromosome artificiel de levure ; ou un chromosome de vertébré ou des fragments d'ADN.

10. Procédé selon l'une quelconque des revendications qui précèdent, dans lequel le fragment d'ADN est inséré par injection ou autres moyens dans des oeufs fécondés ou des cellules souches embryonnaires.

11. Procédé selon l'une quelconque des revendications qui précèdent, dans lequel au départ l'animal n'est porteur d'aucun matériel génétique codant pour les régions constantes de l'immunoglobuline.

12. Immunoglobuline d'origine étrangère pouvant être obtenue par le procédé selon l'une quelconque des revendications qui précèdent.

13. Animal transgénique non-humain dans la lignée germinale duquel on a inséré un fragment d'ADN qui contient un segment d'acide nucléique codant pour au moins une partie d'une immunoglobuline d'origine humaine incluant un segment de gène codant pour une région de chaînes lourdes d'immunoglobuline humaine, dans lequel ledit segment de gène n'est pas sous une forme entièrement réarrangée, pour que l'ADN soit réarrangé dans l'animal pour coder pour un répertoire d'immunoglobulines avec une ou des parties dérivée(s) de l'ADN inséré et exprimée(s) dans les cellules ou un fluide corporel de l'animal.

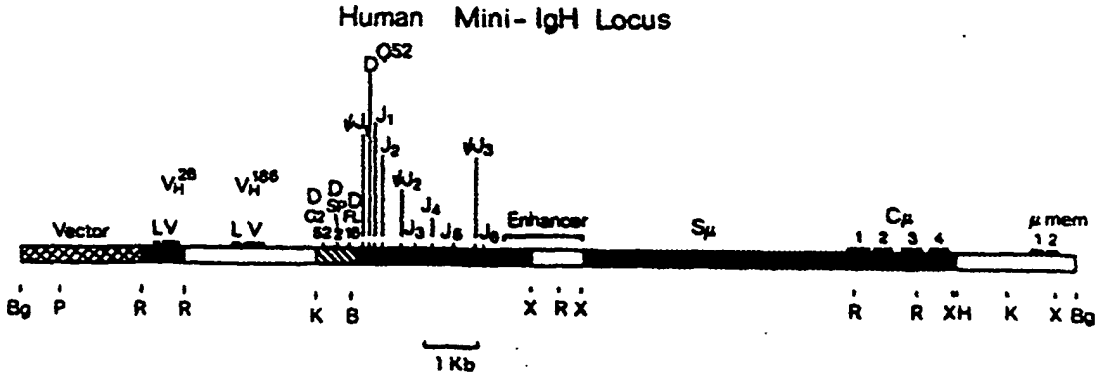

Human Mini-IgH Locus
Vector
LV
LV
Enhancer
Sμ
Cμ
μ mem
Bg P R R K B X R X R R XH K X Bg
1 Kb

Fig. 1

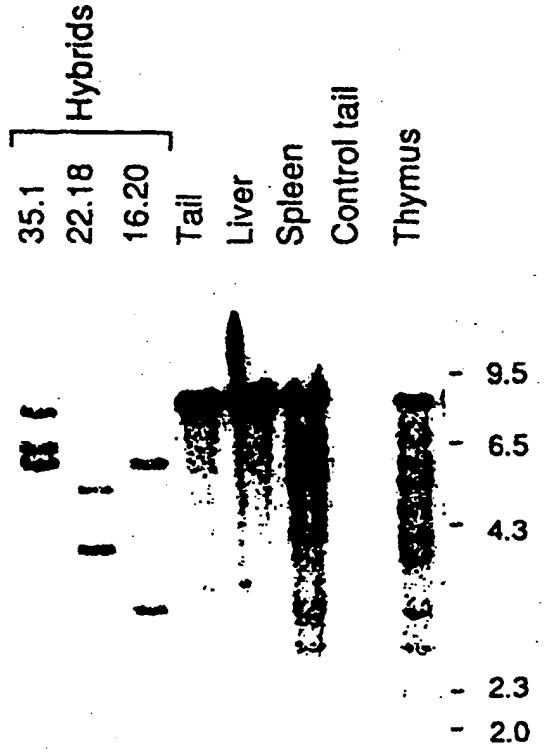

Hybrids
35.1
22.18
16.20
Tail
Liver
Spleen
Control tail
Thymus
9.5
6.5
4.3
2.3
2.0
EcoRI

Fig. 2

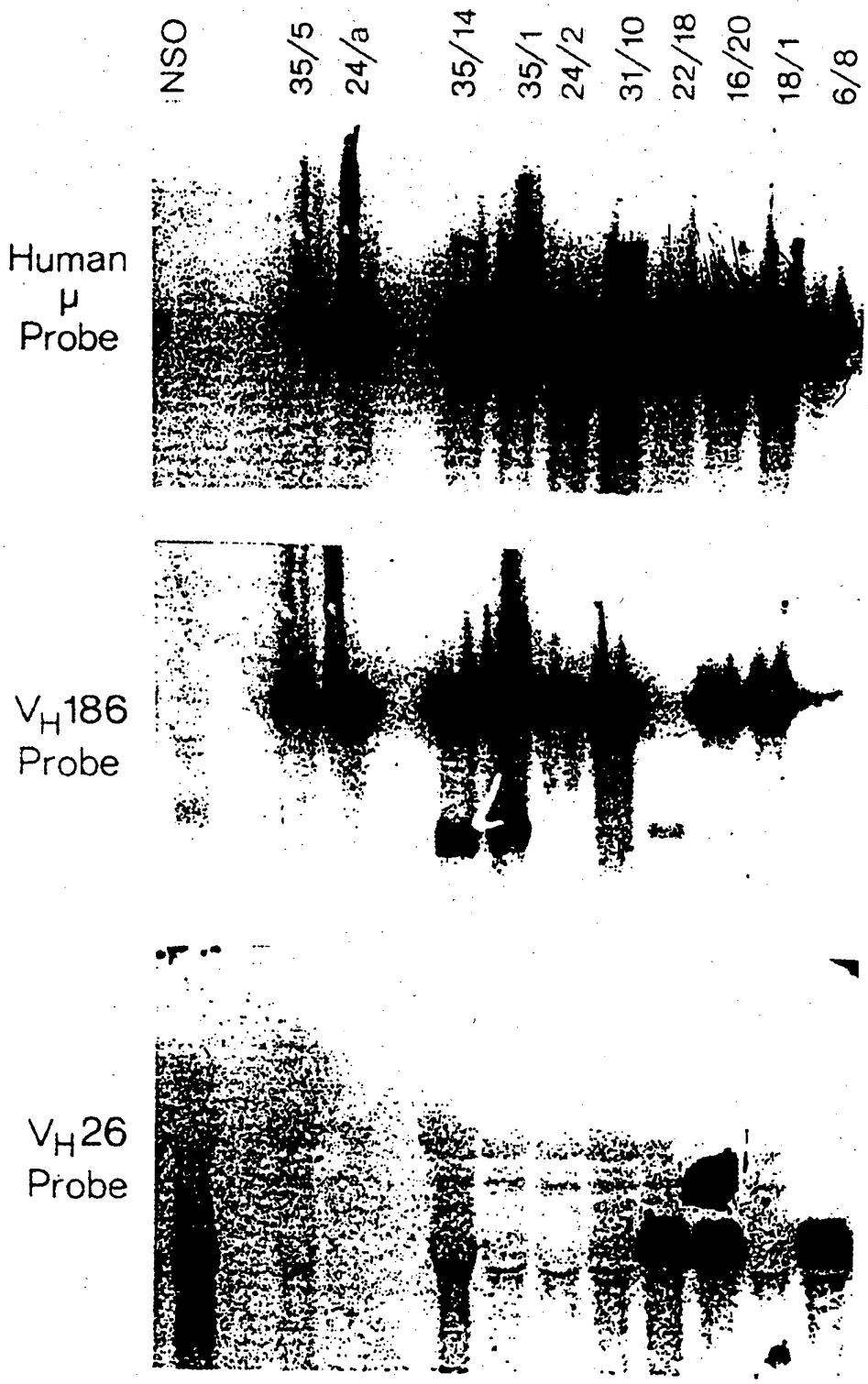

NSO
35/5
24/a
35/14
35/1
24/2
31/10
22/18
16/20
18/1
6/8
Human μ Probe
V_H186 Probe
V_H26 Probe

Fig. 3

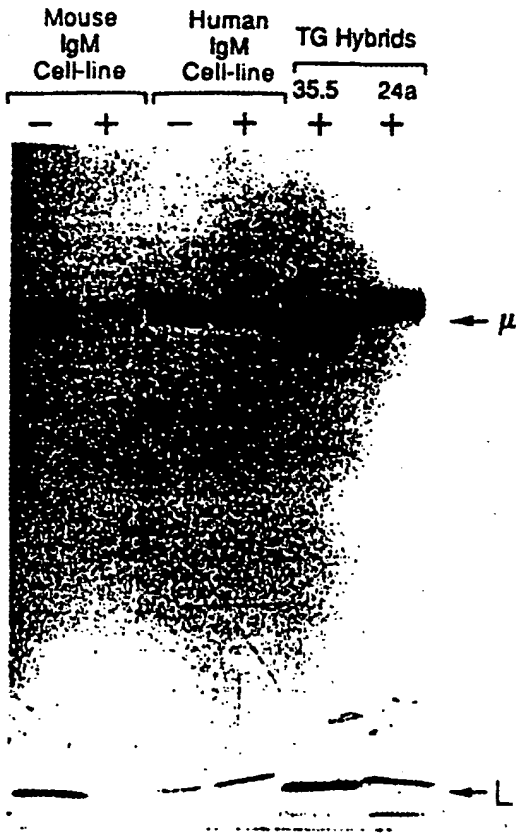

Mouse IgM Cell-line
Human IgM Cell-line
TG Hybrids
35.5
24a
– + – + + +
μ
L

Fig. 4

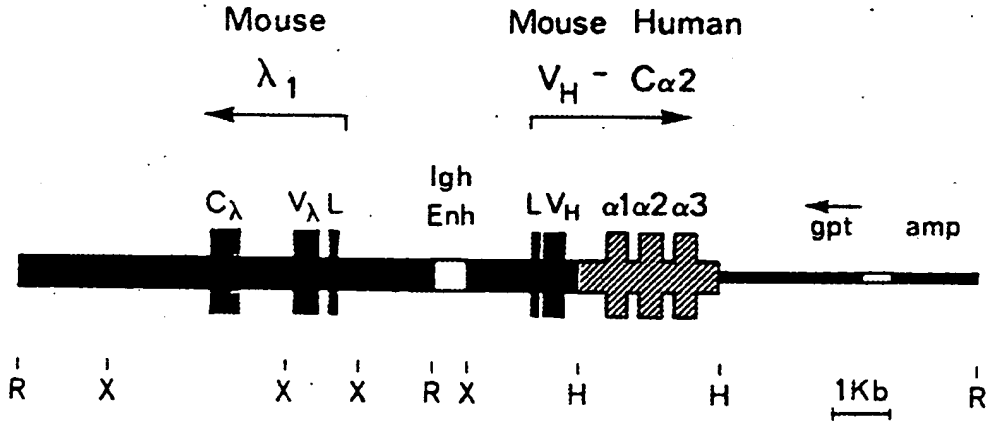

Mouse
λ1
Mouse Human
VH - Cα2
Cλ
Vλ L
Igh Enh
L VH
α1α2α3
gpt
amp
R X X X R X H H 1Kb R

Fig. 5

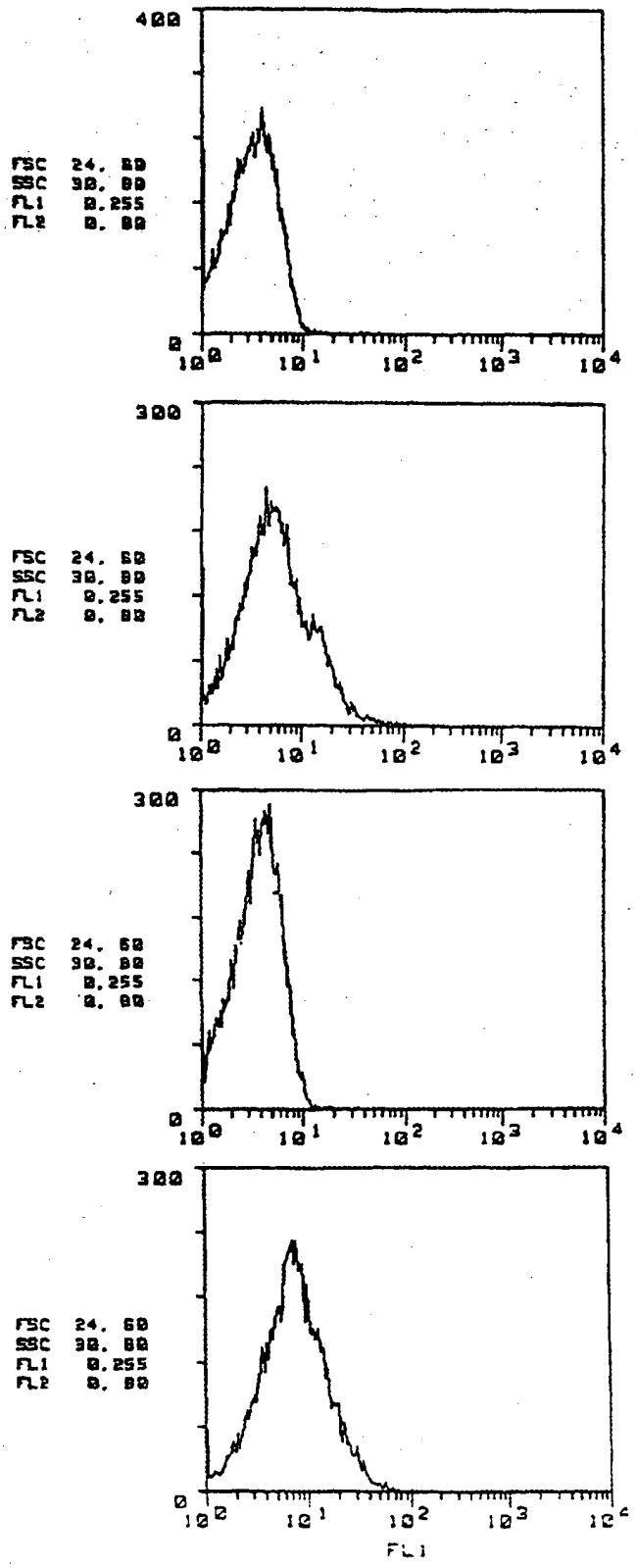

400
FSC 24. 80
SSC 90. 80
FL1 0.255
FL2 0. 80
0
10^0 10^1 10^2 10^3 10^4
A
300
FSC 24. 60
SSC 30. 80
FL1 0.255
FL2 0. 80
0
10^0 10^1 10^2 10^3 10^4
B
300
FSC 24. 60
SSC 90. 80
FL1 0.255
FL2 0. 80
0
10^0 10^1 10^2 10^3 10^4
C
300
FSC 24. 60
SSC 90. 80
FL1 0.255
FL2 0. 80
0
10^0 10^1 10^2 10^3 10^4
FL1
D

Fig. 6

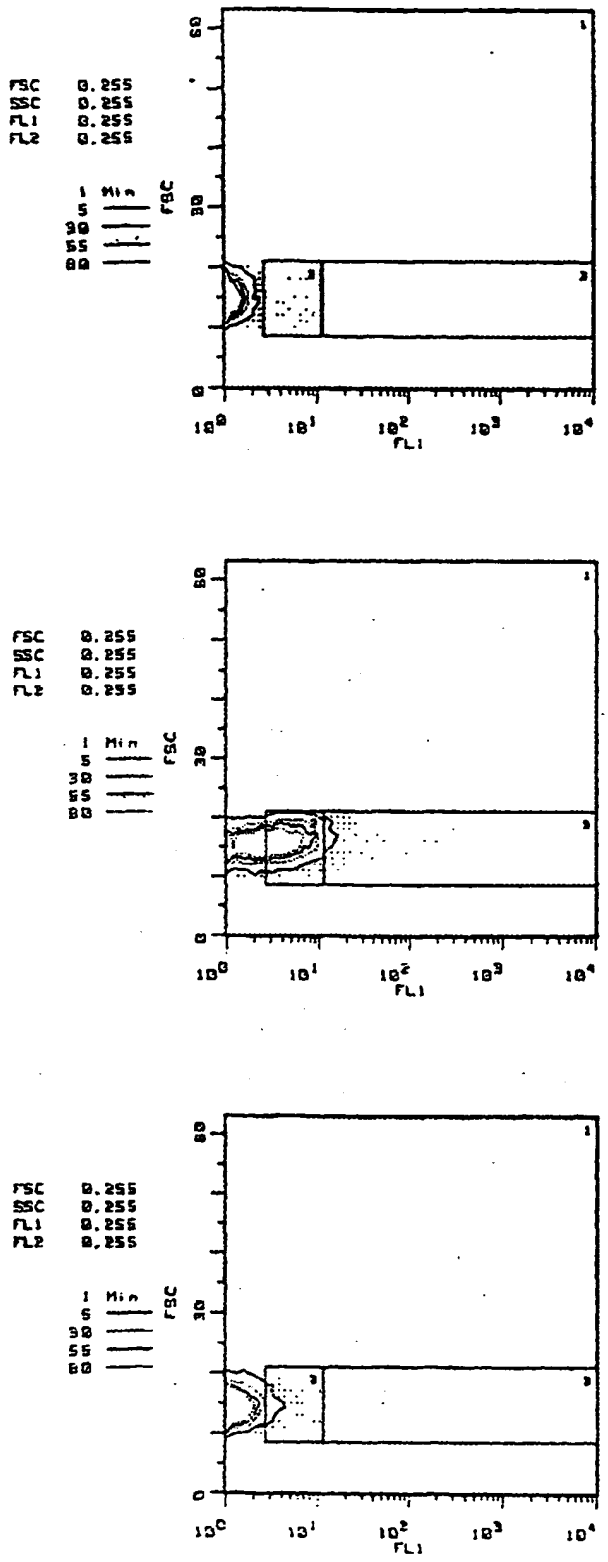
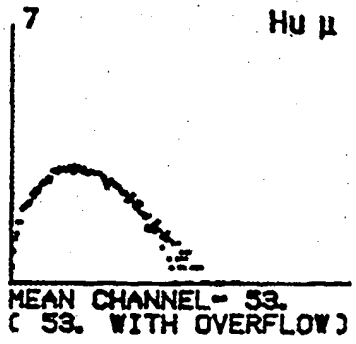
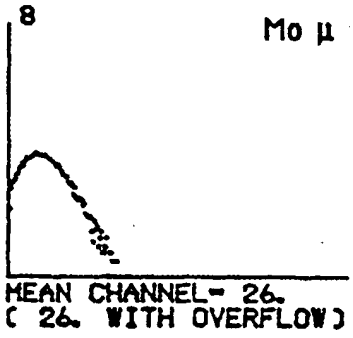


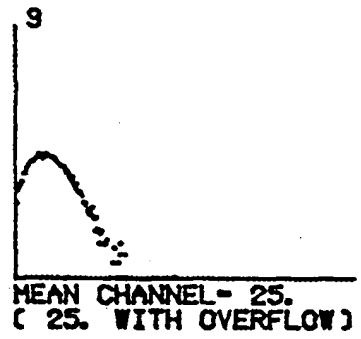
Fig. 7


7
Hu μ
MEAN CHANNEL= 53.
(53. WITH OVERFLOW)

B


8
Mo μ
MEAN CHANNEL= 26.
(26. WITH OVERFLOW)

C


9
MEAN CHANNEL= 25.
(25. WITH OVERFLOW)

A

Fig. 8